IN THE UNITED STATES RECEIVING OFFICE

International Application Number	International Filing Date	International Earliest Priority Date
PCT/US2004/037810	12 November 2004 (12.11.2004)	12 November 2003 (12.11.2003)

TITLE OF INVENTION: SYSTEM FOR TREATING AND PREVENTING BREAST CANCER
APPLICANT FOR DO/US: THERION BIOLOGICS CORPORATION, et al.

Amendments to the Drawings:

The attached sheet of drawing includes changes to Fig. 10. This sheet, which includes Fig. 10, replaces the original sheet including Fig. 10. In Figure 10, a typographical error in the amino acid sequence shown is corrected.

Attachment: Replacement Sheet
Annotated Sheet Showing Changes

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REMARKS

By the present Preliminary Amendment, Applicant has amended the specification to add the cross-reference heading and paragraph. As such, this amendment does not introduce new matter, and its entry is respectfully requested.

Applicants have amended claims 2, 3, 6, 11, 14, 17, and 21 to remove multiple dependences. As such, these amendments do not introduce new matter, and their entry is respectfully requested.

Applicants have added new claims 25-43. Support for the amendments can be found throughout the specification, and particularly, for example, at pages 17-21 and Figures 7-12. Accordingly, the new claims do not introduce new matter, and their entry is respectfully requested.

Applicants submit a replacement Figure 10 to replace the original Figure 10 to correct a typographical error in the amino acid sequence shown in Figure 10. Applicants submit that the replacement Figure 10 does not introduce new matter and should be entered for the following reasons.

The amino acid sequence represents the change of "n" to --d-- as is shown by strikethrough of "n" and underlining of "d" in the following:

"nsnpvededavaltcepeiqnttylwwvnnqslpvsprlqlsndnrtltllsvtrndvgpyecgiqnelsvdhsdpvilnvly
gpddptispsytyyrpgvnlslschaasnppaqyswlidgniqqhtqelfisniteknsglytcqannsasghsrttvktitvsa
elpkpsissnnskpvedkdavaftcepeaqnttylwwvngqslpvsprlqlsngnrtltlfnvtrndarayvcgiqnsvsanr
sdpvtldvlygpdtpiisppdssylsg**a**~~n~~**d**lnlschsasnpspqyswringipqqhtqvlfiakitpnngtyacfvsnlatgrn
nsivksitvsasgtspglsagatvgimigvlvgvali"

Applicants submit that it is clear from the specification, that amino acid sequence should be "D" not "N". For example, in paragraph 57, applicants teach that the epitope "CEA-6D" also called "CAP1-6D" binds with enhanced affinity to the receptor and induces CTL *in vitro* more efficiently than the native epitope. At this place in the specification, Applicants cite to Zaremba et al. 1997 Cancer Research publication (abstract is attached as Appendix A). Zaremba specifically teaches that the "YLSGA**N**LNL" sequence of the wild type is replaced by "YLSGA**D**LNL" in the CAP1-6D mutant. Applicants describe in paragraph 58 that one particularly preferred CEA sequence according to the invention is CAP1-6D. Applicants designate SEQ ID NO.: 4 for the CAP1-6D sequence. Further, the nucleic acid sequence of

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Figure 9 (SEQ ID NO.: 3), encodes the amino acid D, not N, at that portion of the sequence (see nucleic acid translation of SEQ ID No. 3 in Appendix B). In light of the above, Applicants submit that the skilled artisan would have known there was an error in the original SEQ ID No.: 4, and hence in original Figure 10. Thus the Replacement Sheet for Figure 10 does not introduce new matter. Accordingly, Applicants respectfully request that the replacement Figure 10 that corrects the typographical error in the original Figure 10 be entered to reflect the correct sequence.

Applicants did not submit an original sequence listing with the IB. Applicants are submitting herewith said original sequence listing to perfect the application. However, this original sequence listing has an error; therefore, we are also submitting a Substitute Sequence Listing to replace the original sequence listing, which shows the corrected amino acid sequence (SEQ ID No.: 4) present in on the Replacement Sheet for Figure 10. The correction reflects the typographical correction as described, *supra.* Applicants respectfully request entry of the Substitute Sequence Listing.

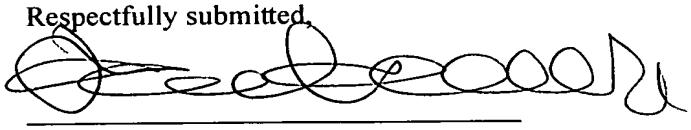
In the event that there are any questions relating to this Amendment or to the application in general, it is kindly requested that the Examiner contact the undersigned attorney concerning the same to expedite prosecution of this application.

Entry of the foregoing and prompt and favorable consideration of the subject application on the merits are respectfully requested.

Fee deficiencies may be charged and overpayments credited to the NIXON PEABODY LLP Deposit Account No. 50-0850.

Date: May 11, 2006

Respectfully submitted,



Ronald I. Eisenstein (Reg. No. 30,628)
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Boston, MA 02110
(617) 345-6054

APPENDIX A

1: Cancer Res. 1997 Oct 15;57(20):4570-7.

Identification of an enhancer agonist cytotoxic T lymphocyte peptide from human carcinoembryonic antigen.

Zaremba S, Barzaga E, Zhu M, Soares N, Tsang KY, Schlom J.

Laboratory of Tumor Immunology and Biology, Division of Basic Sciences, National Cancer Institute, Bethesda, Maryland 20892-1750, USA.

A vaccination strategy designed to enhance the immunogenicity of self-antigens that are overexpressed in tumor cells is to identify and slightly modify immunodominant epitopes that elicit T-cell responses. The resultant T cells, however, must maintain their ability to recognize the native configuration of the peptide-MHC interaction on the tumor cell target. We used a strategy to enhance the immunogenicity of a human CTL epitope directed against a human self-antigen, which involved the modification of individual amino acid residues predicted to interact with the T-cell receptor; this strategy, moreover, required no prior knowledge of these actual specific interactions. Single amino acid substitutions were introduced to the CAP1 peptide (YLSGANLNL), an immunogenic HLA-A2+-binding peptide derived from human carcinoembryonic antigen (CEA). In this study, four amino acid residues that were predicted to potentially interact with the T-cell receptor of CAP1-specific CTLs were systematically replaced. Analogues were tested for binding to HLA-A2 and for recognition by an established CTL line directed against CAP1. This line was obtained from peripheral blood mononuclear cells from an HLA-A2+ individual vaccinated with a vaccinia-CEA recombinant. An analogue peptide was identified that was capable of sensitizing CAP1-specific CTLs 10(2)-10(3) times more efficiently than the native CAP1 peptide. This enhanced recognition was shown not to be due to better binding to HLA-A2. Therefore, the analogue CAP1-6D (YLSGADLNL, Asn at position 6 replaced by Asp) meets the criteria of a CTL enhancer agonist peptide. Both the CAP1-6D and the native CAP1 peptide were compared for the ability to generate specific CTL lines in vitro from unimmunized apparently healthy HLA-A2+ donors. Whereas CAP1 failed to generate CTLs from normal peripheral blood mononuclear cells, the agonist peptide was able to generate

CD8+ CTL lines that recognized both the agonist and the native CAP1 sequence. Most importantly, these CTLs were capable of lysing human tumor cells endogenously expressing CEA. The use of enhancer agonist CTL peptides may thus represent a new efficient direction for immunotherapy protocols.

PMID: 9377571 [PubMed - indexed for MEDLINE]

APPENDIX B

Translate Tool - Results of translation

Please select one of the following frames: of SEQ ID NO:4

5'3' Frame 1

```
atggagtctccctcggcccctccccacagatggtgcatcccctggcagaggctcctgctc
 M  E  S  P  S  A  P  P  H  R  W  C  I  P  W  Q  R  L  L  L
acagcctcacttctaaccttctggaacccgcccaccactgccaagctcactattgaatcc
 T  A  S  L  L  T  F  W  N  P  P  T  T  A  K  L  T  I  E  S
acgccgttcaatgtcgcagaggggaaggaggtgcttctacttgtccacaatctgccccag
 T  P  F  N  V  A  E  G  K  E  V  L  L  L  V  H  N  L  P  Q
catctttttggctacagctggtacaaaggtgaaagagtggatggcaaccgtcaaattata
 H  L  F  G  Y  S  W  Y  K  G  E  R  V  D  G  N  R  Q  I  I
ggatatgtaataggaactcaacaagctaccccagggcccgcatacagtggtcgagagata
 G  Y  V  I  G  T  Q  Q  A  T  P  G  P  A  Y  S  G  R  E  I
atataccccaatgcatccctgctgatccagaacatcatccagaatgacacaggattctac
 I  Y  P  N  A  S  L  L  I  Q  N  I  I  Q  N  D  T  G  F  Y
accctacacgtcataaagtcagatcttgtgaatgaagaagcaactggccagttccgggta
 T  L  H  V  I  K  S  D  L  V  N  E  E  A  T  G  Q  F  R  V
tacccggaactccctaagccttctattagctccaataatagtaagcctgtcgaagacaaa
 Y  P  E  L  P  K  P  S  I  S  S  N  N  S  K  P  V  E  D  K
gatgccgtcgcttttacatgcgagcccgaaactcaagacgcaacatatctctggtgggtg
 D  A  V  A  F  T  C  E  P  E  T  Q  D  A  T  Y  L  W  W  V
aacaaccagtccctgcctgtgtcccctagactccaactcagcaacggaaatagaactctg
 N  N  Q  S  L  P  V  S  P  R  L  Q  L  S  N  G  N  R  T  L
accctgtttaacgtgaccaggaacgacacagcaagctacaaatgcgaaacccaaaatcca
 T  L  F  N  V  T  R  N  D  T  A  S  Y  K  C  E  T  Q  N  P
gtcagcgccaggaggtctgattcagtgattctcaacgtgctttacggacccgatgctcct
 V  S  A  R  R  S  D  S  V  I  L  N  V  L  Y  G  P  D  A  P
acaatcagccctctaaacacaagctatagatcaggggaaaatctgaatctgagctgtcat
 T  I  S  P  L  N  T  S  Y  R  S  G  E  N  L  N  L  S  C  H
gccgctagcaatcctcccgcccaatacagctggtttgtcaatggcactttccaacagtcc
 A  A  S  N  P  P  A  Q  Y  S  W  F  V  N  G  T  F  Q  Q  S
acccaggaactgttcattcccaatattaccgtgaacaatagtggatcctacacgtgccaa
 T  Q  E  L  F  I  P  N  I  T  V  N  N  S  G  S  Y  T  C  Q
gctcacaatagcgacaccggactcaaccgcacaaccgtgacgacgattaccgtgtatgag
 A  H  N  S  D  T  G  L  N  R  T  T  V  T  T  I  T  V  Y  E
ccaccaaaaccattcataactagtaacaattctaacccagttgaggatgaggacgcagtt
 P  P  K  P  F  I  T  S  N  N  S  N  P  V  E  D  E  D  A  V
gcattaacttgtgagccagagattcaaaataccacttatttatggtgggtcaataaccaa
 A  L  T  C  E  P  E  I  Q  N  T  T  Y  L  W  W  V  N  N  Q
agtttgccggttagcccacgcttgcagttgtctaatgataaccgcacattgacactcctg
 S  L  P  V  S  P  R  L  Q  L  S  N  D  N  R  T  L  T  L  L
tccgttactcgcaatgatgtaggaccttatgagtgtggcattcagaatgaattatccgtt
 S  V  T  R  N  D  V  G  P  Y  E  C  G  I  Q  N  E  L  S  V
gatcactccgaccctgttatccttaatgttttgtatggcccagacgacccaactatatct
 D  H  S  D  P  V  I  L  N  V  L  Y  G  P  D  D  P  T  I  S
ccatcatacacctactaccgtcccggcgtgaacttgagcctttcttgccatgcagcatcc
 P  S  Y  T  Y  Y  R  P  G  V  N  L  S  L  S  C  H  A  A  S
aacccccctgcacagtactcctggctgattgatggaaacattcagcagcatactcaagag
 N  P  P  A  Q  Y  S  W  L  I  D  G  N  I  Q  Q  H  T  Q  E
ttatttataagcaacataactgagaagaacagcggactctatacttgccaggccaataac
 L  F  I  S  N  I  T  E  K  N  S  G  L  Y  T  C  Q  A  N  N
tcagccagtggtcacagcaggactacagttaaaacaataactgtttccgcggagctgccc
 S  A  S  G  H  S  R  T  T  V  K  T  I  T  V  S  A  E  L  P
aagccctccatctccagcaacaactccaaacccgtggaggacaaggatgctgtggccttc
```

```
  K  P  S  I  S  S  N  N  S  K  P  V  E  D  K  D  A  V  A  F
acctgtgaacctgaggctcagaacacaacctacctgtggtgggtaaatggtcagagcctc
  T  C  E  P  E  A  Q  N  T  T  Y  L  W  W  V  N  G  Q  S  L
ccagtcagtcccaggctgcagctgtccaatggcaacaggaccctcactctattcaatgtc
  P  V  S  P  R  L  Q  L  S  N  G  N  R  T  L  T  L  F  N  V
acaagaaatgacgcaagagcctatgtatgtggaatccagaactcagtgagtgcaaaccgc
  T  R  N  D  A  R  A  Y  V  C  G  I  Q  N  S  V  S  A  N  R
agtgacccagtcaccctggatgtcctctatgggccggacacccccatcatttccccccca
  S  D  P  V  T  L  D  V  L  Y  G  P  D  T  P  I  I  S  P  P
gactcgtcttacctttcgggagcggacctcaacctctcctgccactcggcctctaaccca
  D  S  S  Y  L  S  G  A  D  L  N  L  S  C  H  S  A  S  N  P
tccccgcagtattcttggcgtatcaatgggataccgcagcaacacacacaagttctcttt
  S  P  Q  Y  S  W  R  I  N  G  I  P  Q  Q  H  T  Q  V  L  F
atcgccaaaatcacgccaaataataacgggacctatgcctgttttgtctctaacttggct
  I  A  K  I  T  P  N  N  N  G  T  Y  A  C  F  V  S  N  L  A
actggccgcaataattccatagtcaagagcatcacagtctctgcatctggaacttctcct
  T  G  R  N  N  S  I  V  K  S  I  T  V  S  A  S  G  T  S  P
ggtctctcagctggggccactgtcggcatcatgattggagtgctggttggggttgctctg
  G  L  S  A  G  A  T  V  G  I  M  I  G  V  L  V  G  V  A  L
atatag
  I  -
```

nsnpvededavaltcepeiqnttylwwvnnqslpvsprlqlsndnrtltllsvtrndvgpy
ecgiqnelsvdhsdpvilnvlygpddptispsytyyrpgvnlslschaasnppaqyswlid
gniqqhtqelfisniteknsglytcqannsasghsrttvktitvsaelpkpsissnnskpv
edkdavaftcepeaqnttylwwvnqqslpvsprlqlsngnrtltlfnvtrndarayv
cgiqnsvsanrsdpvtldvlygpdtpiisppdssylsgaylnlschsasnpspqyswrin
gipqqhtqvlfiakitpnnngtyacfvsnlatgrnnsivksitvsasgtspglsagatvg
imigvlvgvali

d

FIGURE 10

AMINO ACID SEQUENCE OF HUMAN wCEA(6D), SEQ. ID. NO: 4

ANNOTATED SHEET SHOWING CHANGES